

Evidence for Four-Component Close-Range Sex Pheromone in the Parasitic Wasp *Glyptapanteles flavicoxis*

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Received: 6 November 2005 / Revised: 9 February 2006 /
Accepted: 17 February 2006 / Published online: 23 May 2006
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Abstract Females of the parasitic wasp *Glyptapanteles flavicoxis* (Hymenoptera: Braconidae) deposit a close-range sex pheromone from their abdominal tip that attracts conspecific males and elicits wing-fanning behavior. In this study, we isolated the pheromone components and determined their role in the males' behavior. In coupled gas chromatographic-electroantennographic detection (GC-EAD) analyses of the females' body extract, four components (below GC detection) elicited strong responses from male antennae. Monitored by GC-EAD, the components were separated by flash silica gel and high-performance liquid chromatography. Y-tube olfactometer experiments with one or more components revealed that all are necessary to elicit short-range attraction and wing-fanning responses by males. These components remained below detection threshold of the mass spectrometer (~10 pg) even when 4500 female equivalents were analyzed in a single injection, which attests to the potency of the pheromone and the insects' sensitivity to it.

Keywords *Glyptapanteles flavicoxis* · *Lymantria dispar* · Hymenoptera · Braconidae · Parasitoid · Close-range sex pheromone · Wing fanning

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Introduction

Sex pheromones in hymenopteran parasitic wasps are typically produced by females. They have been reported in seven families [Aphelinidae, Chalcididae, Cynipidae, Pteromalidae, Scelionidae, Braconidae, and Ichneumonidae (Kainoh, 1999)], but have been identified in only a few species, including *Itoplectis conquisitor* (Robacker and Hendry, 1977), *Syndipnus rubiginosus* (Eller et al., 1984), *Macrocentrus grandii* (Swedenborg and Jones, 1992a,b), *Ascogaster reticulatus* (Kainoh et al., 1991), *Cardiochiles nigriceps* (Syvertsen et al., 1995), and *Ascogaster quadridentata* (DeLury et al., 1999).

In the Braconidae, sex pheromones have been reported in *Opius alloeus* (Boush and Baerwald, 1967), *Apanteles medicaginis* (Cole, 1970), *Apanteles medicaginis glomeratus* (Obara and Kitano, 1974), *Apanteles medicaginis melanoscelus* (Weseloh, 1976, 1980), *Cotesia rubecula* (Field and Keller, 1994), *Cotesia flavipes* (Kimani and Overholt, 1995), *Praon volucre* (Nazzi et al., 1996), and *Fopius arisanus* (Quimio and Walter, 2000). Most are long-range attractants.

Substrate-borne sex pheromones in parasitoids are rare. Female *Aphelinus asychis* (Hymenoptera: Aphelinidae) appear to have a trail pheromone, but do not exhibit specific trail-marking behavior (Fauvergue et al., 1995). In *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae), a substrate-borne pheromone induces male searching in an area previously explored by females and attracts males from short distance (Pompanon et al., 1997). Female *As. reticulatus*, egg-larval parasitoids of the smaller tea tortrix, *Adoxophyes* sp., employ short-range pheromones that activate searching by males and increase the probability of mating (Kamano et al., 1989).

Some parasitic wasps have multiple-component pheromones. For example, male *M. grandii* are attracted to the female-produced components (Z)-4-tridecenal and (Z,Z)-9,13-heptacosadiene (Swedenborg and Jones, 1992a,b). The behavioral activity of both compounds is enhanced by (3R,5S,6R)-3,5-dimethyl-6-(methyl-ethyl)-3,4,5,6-tetrahydropyran-2-one as a third component that is biosynthetized in mandibular glands of both males and females (Swedenborg et al., 1993). In the ichneumonid *Eriborus terebrans*, the nonpolar pheromone component by itself is inactive, but when added to the polar component provokes the male's behavioral response (Shu and Jones, 1993).

Glyptapanteles flavicoxis (Hymenoptera: Braconidae) is a gregarious, koinobiont endoparasitoid of larval Indian gypsy moth, *Lymantria obfuscata* (Lepidoptera: Lymantriidae) (Marsh, 1979). In 1981, it was imported from India and released into North America as a potential biological control agent for larvae of the European gypsy moth, *Lymantria dispar* (Krause et al., 1990). Female *G. flavicoxis* press their abdominal tip to the substrate, apparently depositing pheromone that elicits wing fanning by males (J. Fuest, personal communication). This interpretation of the females' behavior is supported by reports of abdominal pheromone glands in other braconid females, including *Ap. glomeratus* (Tagawa, 1977), *Ap. melanoscelus* (Weseloh, 1980), *Ap. plutellae*, *Ap. liparis*, *Ap. baoris*, *Ap. ruficrus*, and *Ap. kariyai* (Tagawa, 1983). Female *G. flavicoxis* also emit an airborne component (ethyl dodecanoate) that, by itself, is not effective in attracting conspecific males (J. Fuest, personal communication).

Our objectives were to investigate whether female *G. flavicoxis* use sex pheromone components, and, if so, to isolate them and determine their behavioral role.

Methods and Materials

Experimental Insects

The rearing colonies of experimental insects in the Global Forest Quarantine Facility at Simon Fraser University (SFU) were started and augmented with specimens obtained from the Beneficial Insects Introduction Research Laboratory, United States Department of Agriculture, Agricultural Research Service, Newark, Delaware. To facilitate mating in *G. flavicoxis*, 10 females and 30 males were placed in plastic mesh cages ($10 \times 10 \times 6$ cm) (Hu et al., 1986), and provisioned with cotton wicks (1×10 cm; Richmond Dental, Charlotte, NC, USA) soaked in sugar water solution. Oviposition cages ($18 \times 18 \times 12$ cm) contained 10–15 mated females, five *L. dispar* larvae (3–4 instar) (Fuester et al., 1987), and artificial diet for the larvae (Bell et al., 1981). After 1–2 d, parasitized host larvae were removed and placed on artificial diet in plastic cups (192 ml) with tight-fitting paper lids (Sweetheart Plastics, Wilmington, MA, USA). Every second day, larval frass was removed, diet replenished if needed, and parasite cocoons with insects to be used in bioassays transferred individually to capped plastic cups (30 ml) provisioned with sugar-water-soaked cotton wicks. Cocoons of insects to be used for mass rearing were placed in plastic Petri dishes (14 cm diam.) and food provisioned as described above. Rearing took place under a 16L:8D photoregime at 22–25°C and 50–70% RH.

Acquisition of Volatiles

Unmated, 1- to 2-d-old females (5–10) were placed into vertical cylindrical Pyrex glass chambers (10 ID $\times 6$ cm), and were provisioned with a sugar-water-soaked cotton wick. Control chambers contained the same food source but no parasitoids. A water aspirator drew humidified, charcoal-filtered air at a rate of 1.5–2 l/min for 2 d through the chambers and a glass column (14×1.3 cm OD) filled with 150 mg of Porapak Q (50–80 mesh, Waters Associates Inc., Milford, MA, USA). Volatiles were eluted from the Porapak Q volatile traps with redistilled pentane (2 ml). The extract was concentrated under a stream of nitrogen such that 10 µl of extract contained one female hour equivalent (FHE) of volatile acquisition (= amount of volatiles released by one female during 1 hr).

Acquisition of Pheromone Extracts

Females (1- to 3-d-old) were macerated in vials containing hexane (ca. 10 µl per female) placed on dry ice. The extract was kept at room temperature for ~15 min. The supernatant was withdrawn, filtered through a small amount of glass wool in a pipette, and quantified to determine the volume representing one female body extract (FBE).

Video-recording of Trail-following Behavior by Males

To test the hypothesis that males follow a pheromone trail, their behavioral response was video recorded (Sony Digital Video Camera Recorder, DCR-VX 1000). Into each of 10 Pyrex glass dishes (9×2 cm high), 1 FBE was pipetted in trail-like pattern (Fig. 1). Additional 10 Pyrex glass dishes served as a control stimulus, with solvent

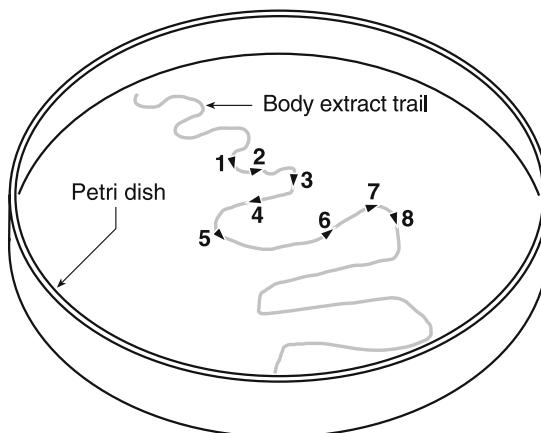
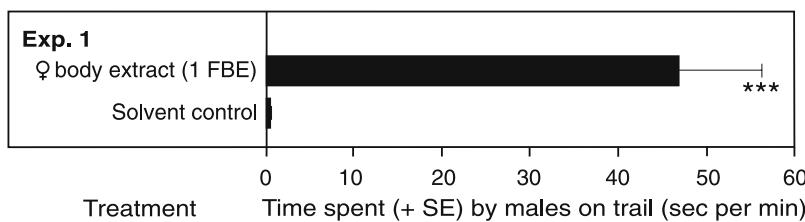


Fig. 1 (Top) Time spent by male *G. flavicoxis* ($n = 10$) on line drawings of a trail treated with one female body extract equivalent or a solvent control. Single-factor analysis of variance, $P < 0.05$. (Bottom) Representative example of “trail-following behavior” by a male (depicted as arrow head), with the position recorded every 2 sec

applied in the same way as the treatment. After the solvent had evaporated (~10 sec), a virgin 1- to 3-d-old male was released and video-recorded for 5 min. Recordings were analyzed for the time a male had spent on the trail and for other characteristic behavioral responses, such as wing fanning.

Y-tube Olfactometer Bioassays

All experiments were conducted during hours 2 to 6 of the insects’ photophase (16L:8D). Anemotactic responses of males to odor sources were tested in vertical Pyrex glass Y-shaped olfactometers (stem: 20 × 2.5 cm ID; side arms at 120°: 18 cm long) positioned vertically 15 cm below a light source, consisting of one tube of fluorescent “daylight” (F40DX, H118; Osram Sylvania Ltd., Ontario, Canada) and one tube of “wide-spectrum grow light” (F40GRO/WS, H658; Osram Sylvania Ltd., Ontario, CA).

Treatment or control (solvent) stimuli were pipetted on white strips of paper (15 × 1 cm) placed in side arms of the Y-tube (experiments 1–28) or on filter paper discs (4.3 cm diam., Whatman No. 1, Whatman International Ltd., Maidstone, England, UK) placed near the orifice of side arms (experiments 29 and 30).

In experiment 31, two live 2- to 3-d-old females served as a test stimulus. They were transferred 10–15 min before experimental replicates into mesh-covered glass

tubes (6×2 cm ID) and provisioned with a sugar-water-soaked cotton wick. Treatment and control tubes (lacking females) were placed at the orifice of side arms of the Y-tube olfactometers.

In all experiments, a water aspirator drew air at ~ 1 l/min through the Y-tube to test anemotactic responses of parasitoids released individually into the stem of the Y-tube. An insect was classed a responder when it traversed the entire paper strip up to the orifice of the side arm within 10 min (experiments 1–28) or contacted the filter paper discs (experiments 29 and 30), or glass tube housing two females (experiment 31). All others were classified as nonresponders. For each replicate, a new insect, paper strip, filter paper disc, and clean (Sparkleen-washed and oven-dried) Y-tube or glass tube were used, with test stimuli randomly assigned to side arms.

To compare the attractiveness of test stimuli most rigorously, two to four experiments were often run in parallel over 2–4 d, alternating between replicates for each experiment. To gauge the relative attractiveness of two or more test stimuli, parallel experiments proved to be more effective than head-to-head comparisons of stimuli in the same Y-tube olfactometer.

Experiment 1 tested the “trail-following[^] response by males. Experiments 2 and 3 determined whether the females’ body extract in combination with the females’ effluvia, or synthetic effluvium component ethyl dodecanoate, were similarly effective in attracting males. Experiments 4 and 5 determined whether males or females respond to the pheromone. Experiments 6–8 explored the relative attractiveness of body extract, ethyl dodecanoate, or both.

Experiments 9–12 tested whether silica fraction 4 (containing candidate close-range pheromone components) and female body extract (containing candidate close-range pheromone components plus traces of ethyl dodecanoate and possibly other components) were equally attractive, at a low dose (1 FHE plus 1 FBE) or medium dose (5 FHE plus 5 FBE). Taking into account that silica fraction 4 at the medium dose was effective in attracting males, experiments 13 and 14 retested whether ethyl dodecanoate enhances the attractiveness of silica fraction 4. Although ethyl dodecanoate did not seem critical for male attraction, it was retained in subsequent experiments (15–27, 30) to ensure the best possible response of males to all test stimuli, and to allow the best comparison of results in all experiments.

Experiments 15 and 16 tested silica fraction 4 at the medium dose vs. the combination of all HPLC fractions that contained candidate close-range pheromone components (= effective blend). Considering the strong attractiveness of the effective blend, follow-up experiments 17, 19, 21, and 23 explored whether one or more of the candidate close-range pheromone components 1 (HPLC fractions 25–28), 2 and 4 (HPLC fractions 21–24), or 3 (HPLC fractions 16–20) could be deleted from the effective blend without affecting the males’ attraction or wing-fanning response. Experiments 25–27 tested the males’ attraction and wing-fanning responses to ethyl dodecanoate (experiment 27) alone, or in combination with either the effective blend (experiment 25) or most EAD-active pheromone component 3 (experiment 26).

Placement of test stimuli near (~ 1 cm) the junction of Y-tubes in experiments 1–28 was appropriate to test close-range anemotactic and wing-fanning responses of males, but not suitable to determine whether ethyl dodecanoate, or other female-produced components, might enhance the active space (mate-recruiting distance) of the entire pheromone blend. Thus, final experiments 29–31 tested the response of males to stimuli [silica fraction 4 on filter paper disc (experiment 29); silica fraction 4 plus ethyl dodecanoate on filter paper disc (experiment 30); two caged live females (experiment

31)] that were placed at the orifice of side arms >10 cm apart from the junction of the Y-tube.

Analyses of Pheromone Extracts

Aliquots of 1 FHE or 1 FBE were analyzed by coupled gas chromatographic–electroantennographic detection (GC-EAD) (Arn et al., 1975; Gries et al., 2002), employing a Hewlett Packard (HP) 5890A gas chromatograph equipped with a GC column (30 m × 0.25 or 0.32 mm ID) coated with DB-5, DB-17, DB-210, DB-23, or FFAP (J & W Scientific, Folsom, CA, USA). For GC-EAD recordings, a male's head was severed and placed into the opening of a glass capillary electrode filled with saline solution (Staddon and Everton, 1980). One antenna with its tip removed by spring microscissors (Fine Science Tools Inc., North Vancouver, British Columbia, CA) was placed into the opening of a second (indifferent) electrode.

EAD-active compounds were analyzed by (1) full-scan electron-impact and chemical ionization (CI, acetonitrile) mass spectrometry (MS) with a Varian Saturn 2000 Ion Trap GC-MS fitted with the DB-5 column referred to above; (2) retention index calculations (Van den Dool and Kratz, 1963); and (3) microanalytical treatments (hydrogenation, oxidation, reduction, acetylation, deacetylation) followed by renewed GC-EAD and GC-MS of the extract.

Aliquots of 100 FBEs with EAD-active components were fractionated through silica gel (0.5 g) in a glass column (14 × 0.5 cm ID). After the silica was prerinse with pentane, the extract was applied, allowed to impregnate the silica gel, and then eluted with six consecutive rinses (1 ml each) of pentane/ether, with increasing proportion of ether, as follows: (1) 100:0; (2) 100:0; (3) 90:10; (4) 75:25; (5) 50:50; and (6) 0:100. This procedure generated fractions containing analytes of increasing polarity.

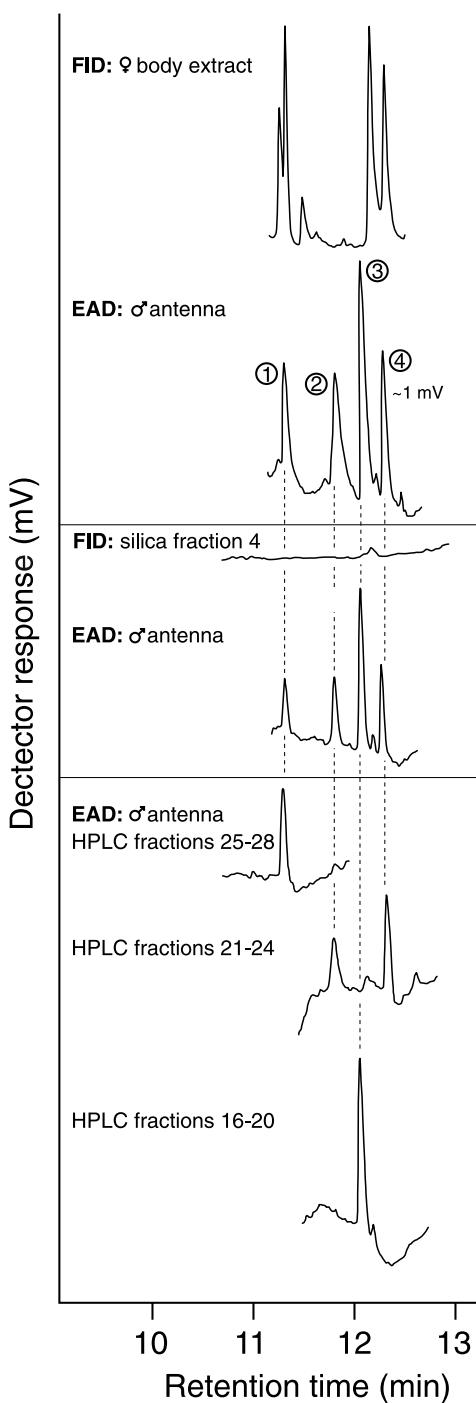
To determine silica fractions with candidate pheromone components, fractions were concentrated to the corresponding number of female equivalents processed in the initial extract and analyzed by GC-EAD, co-injecting as an internal standard ethyl dodecanoate (1 ng), which eluted 4–8 min earlier on the different GC columns than any of the four components. Fractions with more than one EAD-active compound (= candidate pheromone component) were fractionated further into 40 fractions (one fraction/25 sec) by HPLC, followed by renewed GC-EAD analyses of all HPLC fractions. HPLC fractionation employed a Waters LC 626 HPLC equipped with a Waters 486 variable-wavelength UV visible detector set to 210 nm, HP Chemstation software (Rev. A.07.01), and a reverse-phase Nova-Pak C18 column (60 Å, 4 µm; 3.9 × 300 mm) eluted with 1 ml/min of 100% acetonitrile.

Results

In experiment 1, 1 FBE induced wing-fanning and “trail-following behavior[^] by males (Fig. 1). Males also spent more time on trails of body extract of females than on solvent control trails (Fig. 1).

Effluvium (1 FHE) and body extract (1 FBE) of females in combination attracted more males than did the solvent control (Fig. 2, experiment 2). Similarly, ethyl dodecanoate plus female body extract attracted males (Fig. 2; experiments 3, 5, and 8), but not females (Fig. 2, experiment 4). Unlike female body extract, ethyl

Fig. 3 Flame ionization detector (FID) and electroantennographic detector (EAD: male *G. flavicoxis* antenna) responses to aliquots of female body extract (top), silica fraction 4 (middle), and HPLC fractions 16–20, 21–24, and 25–28 (bottom). Chromatography: Hewlett Packard 5890A equipped with a DB-23-coated column (30 m × 0.25 mm ID); linear flow velocity of carrier gas: 35 cm/sec; injector and FID detector temperature: 220°C; temperature program: 1 min at 100°C, 10°C/min to 220°C



dodecanoate by itself failed to attract males or to provoke wing fanning (Fig. 2; experiments 6, 7).

GC-EAD analyses of female body extracts revealed four components that elicited antennal responses from males (Fig. 3; Table 1). Although these components appeared to be abundant in the corresponding flame ionization detector (FID) trace, their mass spectra suggested that they were not pheromone components, but superimposed on them. GC-EAD analyses of all six silica fractions of female body extract revealed that fraction 4 contained the four EAD-active components, and that they indeed occurred below FID detection threshold (Fig. 3).

In Y-tube olfactometers, female body extract (at 1 FBE) combined with ethyl dodecanoate was attractive, whereas silica fraction 4 (at 1 FBE) with ethyl dodecanoate was not (Fig. 4, experiments 9, 10), suggesting that some active material had been lost during fractionation. However, silica fraction 4 at 5 FBE, together with ethyl dodecanoate, attracted males (Fig. 4, experiment 11), indicating that all essential components of the close-range pheromone were present in silica fraction 4. Female body extract at 5 FBE plus ethyl dodecanoate was not attractive (Fig. 4, experiment 12), suggesting that this dose might have exceeded a biologically relevant threshold. In experiments 13 and 14, silica fraction 4 with or without ethyl dodecanoate appeared equally attractive to males.

In GC-EAD analyses of HPLC fractions of silica fraction 4, component 3 was present in fractions 16–20 (elution time: 4–5 min), components 2 and 4 (not separable)

Table 1 Retention indices (relative to alkane standards) of pheromone components 1–4 in body extracts of female *Glyptapanteles flavicoxis* (Fig. 3), and ability of microanalytical treatments of silica or HPLC fractions of body extracts to alter the molecular structure of components 1–4, as determined by the presence or absence of respective antennal responses in GC-EAD recordings of such fractions

GC column	Retention indices of			
	Component 1	Component 2	Component 3	Component 4
DB-5	2068	2089	2083	2108
DB-17	2314	2358	2381	2393
DB-210	2406	2429	2429	2481
DB-23	2583	2658	2700	2731
FFAP	2529	2608	2657	2657
Microanalytical treatments of body extract ^{a,b}	Antennal response in GC-EAD recordings to ^c			
Hydrogenation	Component 1	Component 2	Component 3	Component 4
Absent	Absent	Absent	Absent	Absent
Acetylation	Present	Present	Present	Present
Oxidation (PCC)	Present	Present	Present, but smaller	Present
Reduction (NaBH ₄)	Present	Present	Present	Present
Reduction (LiAlH ₄)	Absent	Absent	Absent	Absent
Deacetylation	Present	Present	Absent	Present

^a Details of these standard treatments are described elsewhere (Huwyler, 1972; Corey and Suggs, 1975; Stanley, 1979; Bjostad et al., 1996; Millar and Haynes, 1998).

^b Each treatment was repeated at least two times with different extracts.

^c Each microtreated extract was tested with at least three male *G. flavicoxis* antennae in GC-EAD recordings.

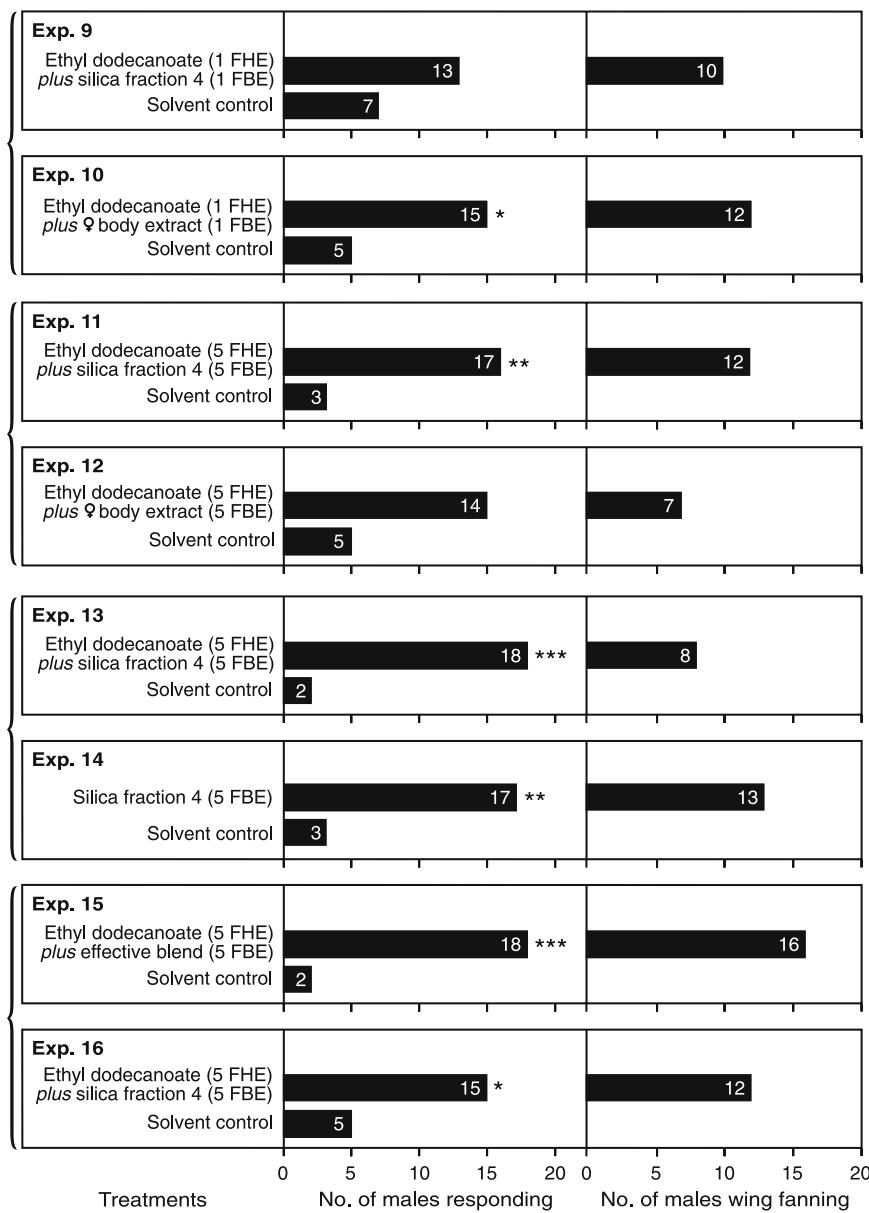


Fig. 4 Number of male *G. flavicoxis* that were attracted, or wing fanned, in response to test stimuli in Y-tube olfactometer experiments 9–16. Abbreviations as in caption of Fig. 2; effective blend = combined HPLC fractions 16–20, 21–24, and 25–28 (see Fig. 3). In each experiment, bars with asterisks (*) indicate a significant response to a particular treatment; heterogeneity χ^2 test with Yates' correction for continuity, treatment vs. control; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Note: (1) Experiments grouped by brackets were run in parallel; (2) one male in experiment 12 did not respond to test stimuli

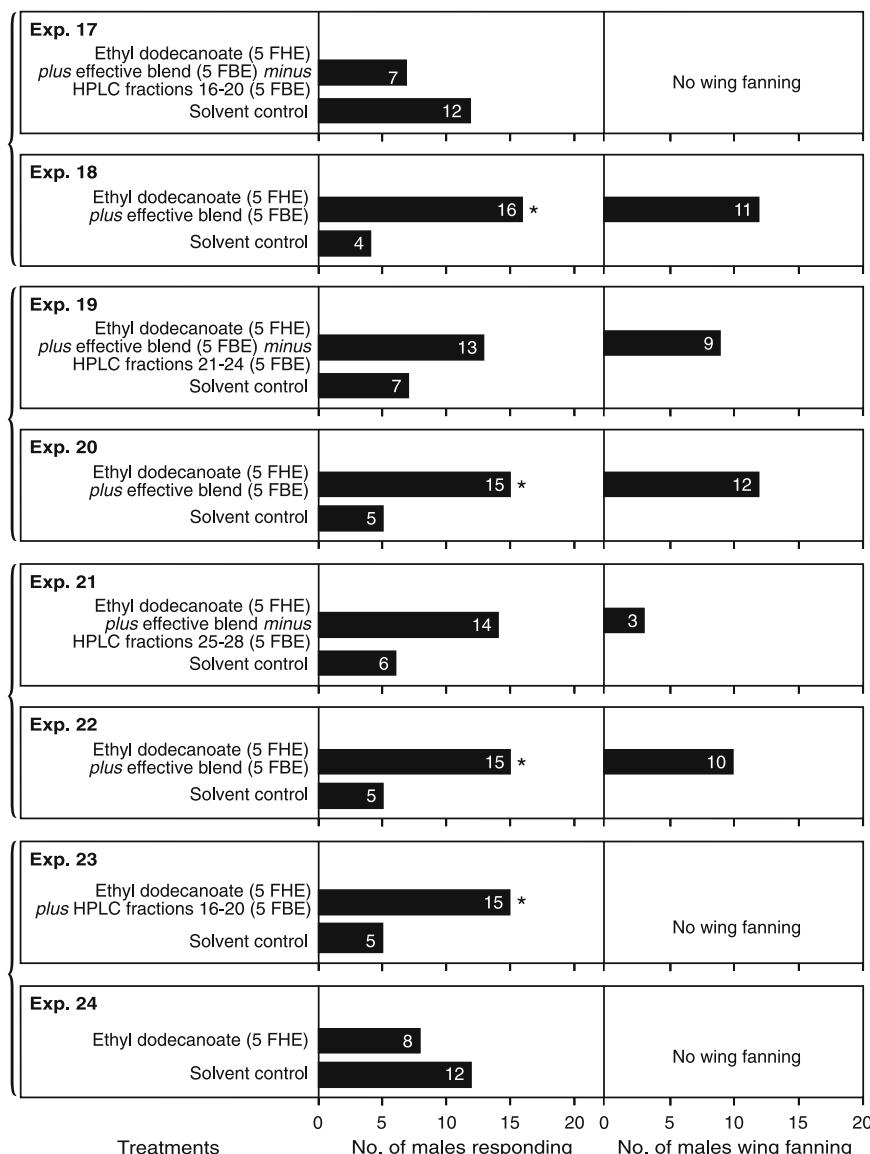


Fig. 5 Number of male *G. flavicoxis* that were attracted, or wing fanned, in response to test stimuli in Y-tube olfactometer experiments 17–24. Abbreviations as in caption of Figs. 2 and 4. In each experiment, bars with asterisks (*) indicate a significant response to a particular treatment; heterogeneity χ^2 test with Yates' correction for continuity, treatment vs. control; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Note: (1) Experiments grouped by brackets were run in parallel; (2) one male in experiment 17 did not respond to test stimuli

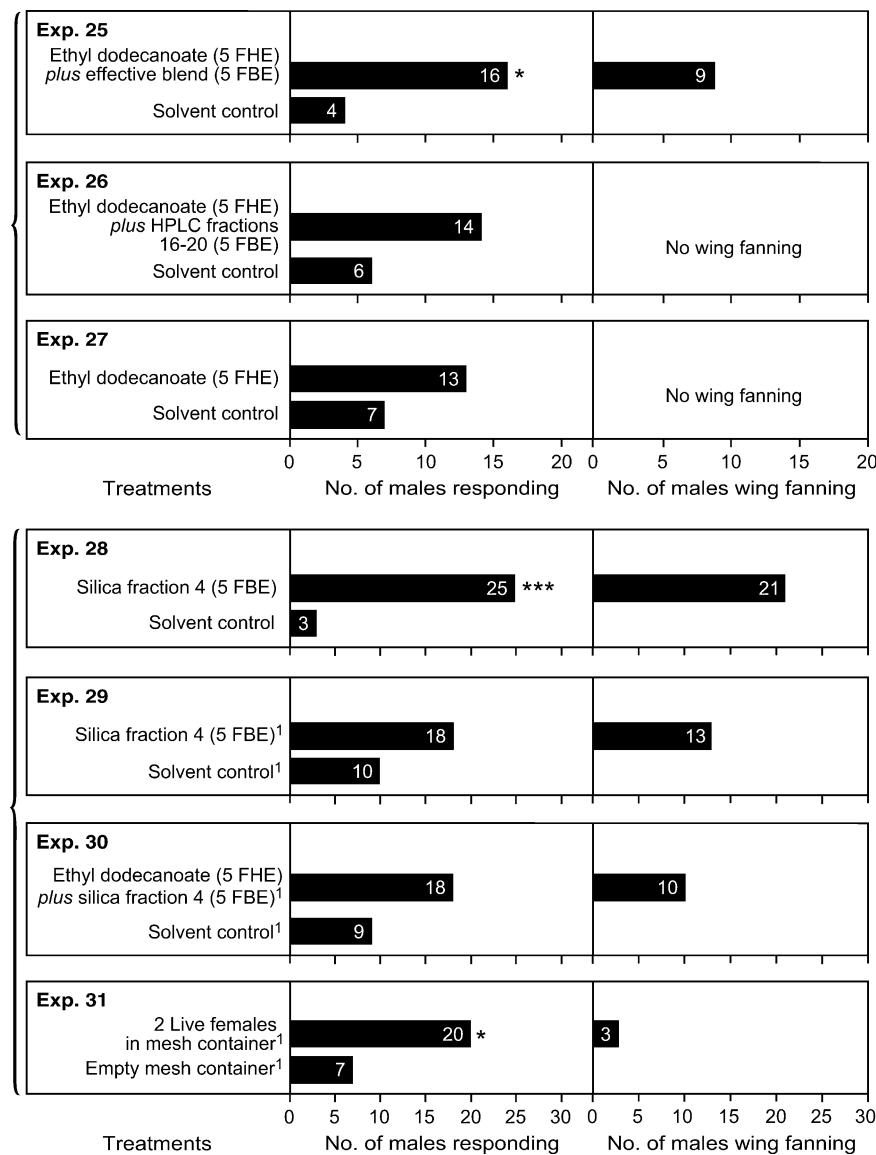


Fig. 6 Number of male *G. flavicoxis* that were attracted, or wing fanned, in response to test stimuli in Y-tube olfactometer experiments 25–31. Abbreviations as in caption of Figs. 2 and 4. In each experiment, bars with asterisks (*) indicate a significant response to a particular treatment; heterogeneity χ^2 test with Yates' correction for continuity, treatment vs. control: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Note: (1) Experiments grouped by brackets were run in parallel; (2) one male in each of experiments 30 and 31 did not respond to test stimuli. ¹Test stimuli placed at the orifice of the Y-tube's side arms

were present in fractions 21–24 (elution time: 5–6 min), and component 1 was present in fractions 25–28 (elution time: 6–7 min) (Fig. 3). In Y-tube olfactometers, all fractions with one or more EAD-active components recombined at 5 FBE, together with ethyl dodecanoate, attracted males (Fig. 4, experiment 15; Fig. 6, experiment 25). This effective blend was no longer attractive to males, when fractions 16–20 (containing component 3), 21–24 (containing components 2 and 4), or fractions 25–28 (containing component 1) were lacking (Fig. 5; experiments 17–22). Ethyl dodecanoate by itself, or in combination with HPLC fractions 16–20, failed to consistently attract males or to elicit wing-fanning responses (Fig. 2, experiment 7; Fig. 5, experiments 23, 24; Fig. 6, experiments 26, 27).

In experiment 28 (Fig. 6), silica fraction 4 applied on a paper strip (15×1 cm) in a Y-tube's side arm prompted strong anemotactic and wing-fanning responses by males (see also experiment 14), but failed to do so, with or without ethyl dodecanoate, when pipetted on a filter paper disc (4.3 cm diam.) at a side arm's orifice in parallel experiments 29 and 30. In contrast, two live females caged at a side arm's orifice were significantly attractive to males (experiment 31).

Discussion

Our data support the conclusion that female *G. flavicoxis* use a four-component pheromone blend that provokes strong close-range anemotactic attraction and wing-fanning responses by conspecific males (Fig. 4, experiment 14; Fig. 6, experiment 28). Response of males but not females to the pheromone (Fig. 2; experiment 4, 5) indicates that it is a sex rather than aggregation pheromone. Failure of these four components to attract males over a distance of 10 cm (Fig. 6, experiment 29), coupled with attraction of males to live females over the same distance (Fig. 6, experiment 31), suggests that females use one or more additional pheromone components for long-range attraction of males. Similarly complex sexual communication has been reported for the parasitic wasp *Aphidius nigripes* (Hymenoptera: Aphidiidae) (McNeil and Brodeur, 1995; Marchand and McNeil, 2000). Body extracts of females provoked wing fanning but not upwind flight by males, suggesting that female *A. nigripes* use both short- and long-range pheromone components.

Ethyl dodecanoate in the effluvia of female *G. flavicoxis* was a potential long-range pheromone component, but it did not affect the males' behavioral response in our experiments (Fig. 4, experiments 13, 14; Fig. 6, experiments 29, 30), and thus cannot be considered a pheromone component.

Video footage (graphical illustration not shown) revealed that females deposit, and males respond to, pheromone on substrate. It is, however, not likely that females deposit a continuous trail, as bioassayed in experiment 1. Males of the braconid *As. reticulatus* respond sporadically to substrate that females have frequented before, suggesting that females deposit traces rather than trails of pheromone (Kamano et al., 1989). Similarly, in *G. flavicoxis*, substrate-borne pheromone may signal the presence of, rather than provide long-range directional cues toward, females (Fig. 6; experiments 29, 30).

Intriguingly, the close-range pheromone blend of *G. flavicoxis* is bifunctional, also eliciting wing-fanning responses by males. The males' strong wing-fanning response, however, was dependent upon their close distance to the pheromone source (e.g., Fig. 4, experiment 14; Fig. 6, experiment 28). Even caged live females (and their

potential pheromone depositions on substrate) that remained inaccessible to males hardly elicited wing-fanning responses (Fig. 6, experiment 31). A strong wing-fanning response was also dependent upon the composition of the pheromone blend. It required the presence of component 3 and component(s) 1, or 2 and 4 (Fig. 5).

Wing fanning has been interpreted as a behavior that facilitates the males' orientation toward females. As demonstrated with fine chalk dust in the ichneumonid *Campoletis sonorensis*, wing fanning pulls air from front to rear, allowing directional orientation of males toward females (Vinson, 1972). This interpretation, however, does not explain completely why male *G. flavicoxis* were so discerning in their wing-fanning response to test stimuli (Fig. 5). Males wing fanned mostly in the presence of the complete pheromone blend, suggesting that they were motivated more by the quality of the female-produced signal than prospects of improved anemotactic orientation toward females. If true, the males' wing fanning could produce sound, possibly so specific that the female could use it to recognize conspecific males and discern between prospective mates (Sivinski and Webb, 1989).

Identification of the close-range sex pheromone components was attempted but failed despite the large sample size (4500 FE) that was analyzed. Nonetheless, numerous microanalytical treatments of, and electrophysiological recording with, pheromone extract (Table 1) suggested that all close-range sex pheromone components are unsaturated molecules of medium polarity, most likely esters. That these compounds remained below detection threshold of the mass spectrometer (~10 pg) even when 4500 FE were analyzed in a single injection, attests to the potency of the pheromone and the insects' sensitivity to it. Alternatively, the components are heat labile (as suggested by an anonymous reviewer), and defy identification by techniques involving gas chromatography.

Acknowledgments We thank Carl Lowenberger for review of the manuscript, Susan Barth, Kenneth Swan, Philip Taylor, and Roger Fuester for supplying experimental insects, Bob Birtch for graphical illustrations, and two anonymous reviewers and Jeremy McNeil for constructive comments. This research was supported, in part, by a graduate fellowship from Simon Fraser University to A.D. and by a Discovery Grant and Industrial Research Chair from the Natural Sciences and Engineering Research Council of Canada (NSERC) to G.G., with Phero Tech. Inc., SC Johnson Canada, and Global Forest as industrial sponsors. Experimental insects were maintained in SFU's Global Forest Quarantine Facility, construction of which was completed through financial support from Global Forest (GF-18-2000-SFU-8).

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